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Vitrification-Based Cryopreservation of Phalaenopsis cornu-Cervi (Breda) Blume & Rchb. f. Protocorms

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Abstract : Protocorms of Phalaenopsis cornu-cervi (Breda) Blume & Rchb. f. were successfully cryopreserved using a vitrification method. Two-month old protocorms at GI 4 stage were precultured in liquid MS medium supplemented with different concentrations of sucrose (0.3, 0.5, 0.7, 0.9 and 1.2 M) at 25±1°C for 2 days on an orbital shaker at 110 rpm. The protocorms were treated with loading solution (2 M glycerol plus 0.4 M sucrose) for 20 minutes at 25±1°C. Then, the protocorms were sufficiently dehydrated with vitrification solution (plant vitrification solution 2, PVS2) for various times (0, 30, 60, 90 and 120 minutes) at 25±1°C and stored in liquid nitrogen for 1 day. After rapid thawing in water bath at 40°C for 2 minutes, the explants were washed by MS liquid medium containing 0.5 ml of 1.2 M sucrose for 20 minutes. The results shown that the protocorms were precultured in liquid MS medium containing 0.5 M sucrose and dehydrated with vitrification solution for 60 minutes had the highest survival percentage of protocorm at 31±1.0 % as measured by Evan's blue. No survival rate of protocorms was found without vitrification treatments.

Keywords : protocorms, cryopreservation, Phalaenopsis cornu-cervi, vitrification
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